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(54) Title: SYNTHETIC IMMUNOACTIVE PEPTIDES HAVING IMMUNOMODULATING AND THERAPEUTIC ACTIVITIES

(57) Abstract

Synthetic peptides which are effective in the therapy of immunodeficiencies, immunosuppression and T-cell subset deviations, and related ailments.

+ DESIGNATIONS OF "SU"

Any designation of "SU" has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.

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Description

Synthetic Immunoactive Peptides Having Immunomodulating and Therapeutic Activities

Technical Field

The present invention relates, generally, to synthetic immunoactive peptides having immunodulating and therapeutic activities and use of such peptides in pharmaceuticals. More particularly, the present invention relates to chemically synthesized immunoactive peptides useful in immunotherapy for regulation of T-cell dependent immunity.

Background Art

It is generally known in the prior art that a great number of disorders in humans, as well as animals, are associated with decreased immunity or immunodeficiency. Various degrees of altered levels of immunity are found in oncologic and hematalogic diseases, aging, etc. As a result of immunological dysfunction, various infections, neoplasias and accerated metastasizing may be observed in persons suffering from such disorders.

The crucial role of thymus-dependent immunity in the function of the immune system is well-established. Recently, a number of biologically active polypeptides have been isolated from the thymus and, at least partially, characterized. Among such polypeptides are various thymosins, thymopoietins and thymic serum factor. These results are detailed in Cardarelli, Nate P., The Thymus in Health and Senescence, CRC Press (1989); Goldstein, Allan L., Thymic Hormones and Lymphokines: Basic Chemistry and Clinical Applications, NY, Plenum Press (1989); and, Gideon Goldstein et al., NY Liss (1987).

Fraction V, the uncharacterized mixture of polypeptides from calf thymus extract, having relatively high amounts of alpha-, beta- and gamma-thymosins and related polypeptides, has proven to be useful from immunomodulation in in vitro and in vivo tests, as summarized in Aiuti, F. and Wigzell, H., Thymus, Thymic Hormones, and T-Lymphocytes, Proceedings of the Serono Symposium, V. 38 (Academic Press 1989.) The potential effectiveness of Fraction V as an immunomodulating drug has been disclosed in H. Strausser, U.S. Patent No. 4,444,757, issued April 24th, 1984.

The clinical use of the foregoing preparations has been limited by:

1. The impossibility of obtaining an adequate quantity for studies, as well as the need for using a significant amount of autological material of thymuses of newborn children:

2. The impossibility of precise biochemical identification of extracts; and,

3. An immune response to the xenogenic or allogenic proteins from thymic extracts.

In summary, the use of purified natural or recombinant polypeptides for immunotherapy is limited by the limited source of biologically active components, as well as the immunological and biochemical difficulties in their purification. Synthetic peptides avoid these drawbacks.

Different immunologically active synthetic peptides, related to either natural thymosins alpha 1, beta 3 and beta 4, as modified sequences, have been disclosed in, for example, S. Wang, U.S. Patent No. 4,116,951, issued September 26th, 1978; T. Low et

al., U.S. Patent No. 4,395,404, issued July 26th, 1983; and, C. Birr et al., U.S. Patent No. 4,612,365, issued September 16th, 1986. Additionally, the active synthetic peptide of thymopoietin has been described in G. Goldstein et al., Science, 1979, 204, p. 1309. This peptide sequence has been disclosed in G. Goldstein et al., U.S. Patent No. 4,190,646, issued February 1980, and modified sequences having similar activity were disclosed in G. Goldstein et al., U.S. Patent No. 4,201,886, issued April 14th, 1981. These peptide sequences induce differentiation of lymphocytes into mature T-cells expressing specific markers, support the delicate equilibrium of helper and suppressor T-cells, stimulate the previously-diminished immune response and suppress excessive autoimmune activity to various auto-antigens.

Nevertheless, the overall biological activities of presently known synthetic peptides have proven to be insufficient for clinical use, as detailed, for example, in B. K. Kantharia et al., "Thymopentin (TP-5) in the treatment of Rhumatoid arthritis," Br. J. Rheumatol, 1989, 28(2), pp. 118-123; J. A. Hansen,

J. E. Sanders, R. Stuart, "Use of thymic grafts of thymic factors to augment immunologic recovery after bone marrow transplantation: brief report with 2 - 12 years follow-up. Bone marrow transplant, 1980, pp. 424-436.

Pentapeptide sequences with thymopoietin-like activity and increased resistance to enzymatic degradation in biological fluids were created and disclosed in G. Goldstein et al., U.S. Patent No. 4,505,853, issued March 19th, 1985, however, their biological activity was comparable only with activity of parental peptide sequences.

In order to overcome the weak activities of the thymopoietin-derived peptide to the #32-36 sequence, an additional peptide from alpha-1 thymosin was added. This further procedure, however, failed to increase the biological activity of the parent sequences, as detailed in M. Mokotoff et al., "Thymosin-like peptides as potential immunostimulants. Synthesis via the polymeric-reagent method," J. Med. Chem., 1990, 33(1), pp. 354-360.

Additional prior art known to the inventor, but

which has failed to overcome the difficulties encountered in the production of immunotherapeutically active peptides, includes G. Goldstein et al., U.S. Patent No. 4,002,740, issued January 11th, 1977; W. McGregor, U.S. Patent No. 4,082,737, issued April 4th, 1978; G. Goldstein, U.S. Patent No. 4,124,700, issued November 7th, 1978; A. Goldstein et al., U.S. Patent No. 4,297,276, issued October 27th, 1981; C. Birr et al., U.S. Patent No. 4,353,821, issued October 12th, 1982; G. Heavner, U.S. Patent No. 4,369,137, issued January 18th, 1983; B. Horecker, U.S. Patent No. 4,374,197, issued February 15th, 1983; G. Goldstein et al., U.S. Patent No. 4,397,842, issued August 9th, 1983; C. Birr et al., U.S. Patent No. 4,466,918, issued August 21st, 1984; C. Birr et al., U.S. Patent No. 4,470,926, issued September 11th, 1984; A. Felix et al., U.S. Patent No. 4,504,415, issued March 12th, 1985; A. Felix et al., U.S. Patent No. 4,517,119, issued May 14th, 1985; B. Horecker, U.S. Patent No. 4,659,694, issued April 21st, 1987; B. Horecker, U.S. Patent No. 4,716,148, issued December 29th, 1987; and, C. Birr et al., U.S. Patent No. 4,910,296, issued March 20th, 1990.

It is clear that if the difficulties encountered in the production of immunotherapeutically active peptides could be overcome, a major advancement in the treatment of various diseases would be achieved. Thus, a great need exists for the creation of biologically active peptides which can be used for the clinically effective correction of immunodeficiencies and immunosuppressions associated with different pathological conditions.

Disclosure of Invention

It is, therefore, an object of the present invention to provide a series of synthetic peptides which are effective in the therapy of immunodeficiencies, immunosuppression and T-cell subset deviations, and related ailments.

It is an additional object of the present invention to provide a series of synthetic peptides which have a biological activity which is several hundred times more effective than presently known immunologically-active sequences.

It is, yet, a further object of the present invention to provide synthetic peptides which are

non-toxic to humans, even when administered in doses which are several thousand times higher than effective dose levels.

It is an additional object of the present invention to provide a series of synthetic peptides which overcome the disadvantages inherent in the prior art and with similar substances found in nature.

The foregoing and related objects are achieved by linear and cyclized peptide compounds constructed by combination and/or overlapping of the sequences:

A_1B_1 X B_2A_2, A_3B_3 X A_4B_4, B_5A_5 X A_6B_6,
B_7A_7 X B_8A_8, A_9B_9, $A_{10}A_{11}$,
$B_{10}A_{12}$ and/or $B_{11}B_{12}$,

wherein,

X is a hydrophobic or neutral amino acid independently selected from the group consisting of Ala, *D*-Ala, Gly, *D*-Gly, Ile, *D*-Ile, Leu, *D*-Leu, Met, *D*-Met, Phe, *D*-Phe, Pro, *D*-Pro, Sar, *D*-Sar, Ser, *D*-Ser, Tre, *D*-Tre, Trp, *D*-Trp, Val and *D*-Val;

A_1 through A_{12} are, independently, neutral or positively-side-chain-charged amino acids independently selected from the group consisting of Ala, *D*-Ala, Arg, *D*-Arg, Asp, *D*-Asp, Glu, *D*-Glu, Gly, *D*-Gly, His, *D*-His, Ile, *D*-Ile, Leu, *D*-Leu, Met, *D*-Met, Phe, *D*-Phe, Pro, *D*-Pro, dehydro Pro, Sar, *D*-Sar, Ser, *D*-Ser, Tre, *D*-Tre, Trp, *D*-Trp, Val and *D*-Val;

and,

B_1 through B_{12} are, independently, neutral or negatively-side-chain-charged amino acids independently selected from the group consisting of Ala, *D*-Ala, Asp, *D*-Asp, Glu, *D*-Glu, Gly, *D*-Gly, Ile, *D*-Ile, Leu, *D*-Leu, Met, *D*-Met. Phe, *D*-Phe, Pro, *D*-Pro, dehydro Pro, Sar, *D*-Sar, Tre, *D*-Tre, Tyr, *D*-Tyr, Val, *D*-Val, *L*-2- amino-glutaryl, *D*-2-aminoglutaryl, *L*-2-aminoadiphyl, *D*-2-aminoadiphyl, *L*-2-aminopimelyl and *D*-2-aminopimelyl.

The foregoing peptides, in accordance with the present invention, may be acylated and/or amidated.

The foregoing generic formula includes the following sequences of amino acids in accordance with the foregoing definitions for "A," "B" and "X";

1. $A_1 A_2 B_2 XA_3 B_3$
2. $A_1 A_2 XB_2 A_2 A_3$
3. $A_1 B_1 XB_2 A_2 A_3$
4. $B_1 A_1 XB_2 A_2 A_3$
5. $B_1 B_2 A_2 XA_3 B_3$
6. $B_1 B_2 A_2 XB_3 A_3$
7. $A_1 B_1 XA_2 B_2 B_3$
8. $B_1 A_1 XA_2 B_2 B_3$
9. $A_1 B_2 A_2 XA_3 B_3$
10. $A_1 B_2 A_2 XB_3 A_3$
11. $A_1 B_1 XB_2 A_2 B_3$
12. $B_1 A_1 XB_2 A_2 B_3$
13. $B_1 A_2 B_2 XB_3 A_3$
14. $B_1 A_2 B_2 XA_3 B_3$
15. $A_1 B_1 XA_2 B_2 A_3$
16. $B_1 A_1 XA_2 B_2 A_3$
17. $A_1 A_2 A_3 B_3 XB_4 A_4$
18. $A_1 A_2 A_3 B_3 XA_4 B_4$
19. $A_1 A_2 B_3 A_3 XA_4 B_4$
20. $A_1 A_2 B_3 A_3 XB_4 A_4$
21. $A_1 B_1 XB_2 A_2 A_3 A_4$
22. $A_1 B_1 XA_2 B_2 A_3 A_4$
23. $B_1 A_1 XA_2 B_2 A_3 A_4$
24. $B_1 A_1 XB_2 A_2 A_3 A_4$
25. $B_1 B_2 A_3 B_3 XB_4 A_4$
26. $B_1 B_2 A_3 B_3 XA_4 B_4$
27. $B_1 B_2 B_3 A_3 XA_4 B_4$
28. $B_1 B_2 B_3 A_3 XB_4 A_4$
29. $A_1 B_1 XB_2 A_2 B_3 B_3$
30. $A_1 B_1 XAB_2 B_3 B_4$
31. $B_1 A_1 XA_2 B_2 B_3 B_4$
32. $B_1 A_1 XB_2 A_2 B_3 B_4$
33. $A_1 B_1 A_2 B_2 XB_3 A_3$
34. $A_1 B_1 A_2 B_2 XA_3 B_3$
35. $A_1 B_1 B_2 A_2 XA_3 B_3$
36. $A_1 B_1 B_2 A_2 XB_3 A_3$
37. $A_1 B_1 XB_2 A_2 A_3 B_3$
39. $A_1 B_1 XA_2 B_2 A_3 B_3$
39. $B_1 A_1 XA_2 B_2 A_3 B_3$
40. $B_1 A_1 XB_2 A_2 A_3 B_3$

41. $B_1 A_1 A_2 B_2 XB_3 A_3$

42. $B_1 A_1 A_2 B_2 XA_3 B_3$

43. $B_1 A_1 B_2 A_2 XA_3 B_3$

44. $B_1 A_1 B_2 A_2 XB_3 A_3$

45. $A_1 B_1 XB_2 A_2 B_3 A_3$

46. $A_1 B_1 XA_2 B_2 B_3 A_3$

47. $B_1 A_1 XA_2 B_2 B_3 A_3$

48. $B_1 A_1 XB_2 A_2 B_3 A_3$

49. $B_1 A_1 A_2 B_2 XB_3 A_3 A_4 B_4$

50. $A_1 B_1 A_2 B_2 XB_3 A_3 B_4 A_4$

51. $B_1 A_1 A_2 B_2 XA_3 B_3 A_4 B_4$

53. $B_1 A_1 B_2 A_2 XA_3 B_3 A_4 B_4$

53. $A_1 B_1 B_2 A_2 XA_3 B_3 B_4 A_4$

54. $A_1 B_1 A_2 B_2 XA_3 B_3 B_4 A_4$

55. $B_1 A_1 B_2 A_2 XB_3 A_3 A_4 B_4$

56. $A_1 B_1 B_2 A_2 XB_3 A_3 B_4 A_4$

57. $B_1 A_1 A_2 B_2 XB_3 A_3 B_4 A_4$

58. $A_1 B_1 A_2 B_2 XB_3 A_3 A_4 B_4$

59. $B_1 A_1 A_2 B_2 XA_3 B_3 B_4 A_4$

60. $A_1 B_1 A_2 B_2 XA_3 B_3 A_4 B_4$

61. $B_1 A_1 B_2 A_2 XA_3 B_3 B_4 A_4$

62. $A_1 B_1 B_2 A_2 XA_3 B_3 A_4 B_4$

63. $B_1 A_1 B_2 A_2 XB_3 A_3 B_4 A_4$

64. $A_1 B_1 B_2 A_2 XB_3 A_3 A_4 B_4$

65. $A_1 B_2 XA_2 A_2 XA_3 B_3$

66. $A_1 B_1 XB_2 A_2 XB_3 A_3$

67. $A_1 B_1 XA_2 B_2 XB_3 A_3$

68. $B_1 A_1 XA_2 B_2 XB_3 A_3$

69. $B_1 A_1 XA_2 B_2 XA_3 B_3$

70. $B_1 A_1 XB_2 A_2 XA_3 B_3$

71. $A_1 B_1 XB_2 A_2 A_3 B_3 XB_4 A_4$

72. $A_1 B_1 XA_2 B_2 A_3 B_3 XA_4 B_4$

73. $B_1 A_1 XA_2 B_2 B_3 A_3 XA_4 B_4$

74. $B_1 A_1 XB_2 A_2 B_3 A_3 XB_4 A_4$

75. $A_1 B_1 XB_2 A_2 A_3 B_3 XA_4 B_4$

76. $A_1 B_1 XA_2 B_2 A_3 B_3 XB_4 A_4$

77. $A_1 B_1 XB_2 A_2 B_3 A_3 XA_4 B_4$

78. $B_1 A_1 XA_2 B_2 A_3 B_3 XB_4 A_4$

79. $A_1 B_1 XB_2 A_2 B_3 A_3 XB_4 A_4$

80. $B_1 A_1 XB_2 A_2 A_3 B_3 XB_4 A_4$

81. $A_1 B_1 XA_2 B_2 B_3 A_3 XB_4 A_4$

82. $B_1 A_1 XB_2 A_2 A_3 B_3 XA_4 B_4$

83. $A_1 B_1 XA_2 B_2 B_3 A_3 XA_4 B_4$

84. $B_1 A_1 XA_2 B_2 A_3 B_3 XA_4 B_4$

85. $B_1 A_1 XA_2 B_2 B_3 A_3 XB_4 A_4$

86. $B_1 A_1 XB_2 A_2 B_3 A_3 XA_4 B_4$

The present invention should be understood as encompassing pharmaceutically acceptable acid or base salts, as well as the free peptides generically described above and which are detailed hereinafter.

For the purposes of achieving the objects of the present invention, amino acid sequence No. 41 provides the most effective peptides. Amino acid sequences Nos. 49 and 59 have also been found to be very effective.

It was surprisingly discovered that the peptides of the invention, as defined above, express immunomodulating activity, with many such peptides expressing a more powerful immunodulating activity than known natural, or naturally modified, amino acid sequences. The compositions to be administered to a patient in the treatment of immunodeficiencies, immunosuppression, T-cell subset deviations and for the enhancement of vaccinations, etc., is to include one or more of the foregoing peptides in combination with an appropriate solubilizer. Suitable additives known to those skilled in the art, e.g., carriers, preservatives and viscosity modifiers, may be added to the compounds of the invention as required.

The synthetic immunologically-active peptides of the present invention are, for example, for parenteral use, direct intranasal, ear, eye, intravaginal or rectal instillation and application to intact or injured conjunctive, mucosa or skin, in order to accomplish the normalization of immune responses. The preparations are to be used either locally or systemically in order to induce immunomodulation, enhance the effect of vaccination and achieve other goals of immunotherapy.

Preferred peptides of the present invention are those wherein:

A_1 through A_n are, independently, Arg, Asn, Gln, Lys, Phe or Val;

B_1 through B_n are, independently, Asp, Glu, Tyr, Phe or Val;

X is Ala, Gly, Ile, Leu, Phe or Val.

Most preferred peptides coming within the scope of the present invention contain either balanced side chain charges of the sequences which are symmetical relative to X or uncompensated opposite side chain

charges relative to X.

For protection against amino- and carboxypeptidases, peptides may be acylated, amidated or cyclized during synthesis.

Examples of peptides falling within the scope of the present invention, which have been found to be particularly effective include:

Arg-Asp-Lys-Asp-Val-Tyr-Arg

Lys-Asp-Lys-Asp-Val-Tyr-Lys

Lys-Glu-Lys-Asp-Val-Tyr-Lys

Arg-Glu-Arg-Asp-Val-Tyr-Arg

Lys-Glu-Leu-Tyr-Arg-Lys-Glu

Lys-Glu-Leu-Glu-Lys-Lys-Glu

Arg-Glu-Leu-Glu-Arg-Arg-Glu

Lys-Asp-Val-Asp-Lys-Lys-Asp

Lys-Asp-Leu-Glu-Lys-Lys-Glu

Tyr-Arg-Lys-Asp-Val-Tyr-Arg-Lys-Glu

Tyr-Arg-Lys-Glu-Leu-Glu-Lys-Lys-Glu

Glu-Lys-Lys-Glu-Leu-Tyr-Arg-Lys-Glu

Tyr-Arg-Lys-Glu-Leu-Tyr-Arg-Glu-Lys

Tyr-Arg-Lys-Asp-Val-Tyr-Arg-Lys-Asp-Val-Tyr-Arg

Glu-Lys-Lys-Glu-Leu-Tyr-Arg-Lys-Glu-Leu-Tyr-Arg

Glu-Arg-Lys-Glu-Leu-Tyr-Arg-Lys-Glu-Leu-Tyr-Arg

Examples of peptides synthesized in accordance with the present invention, which have been found to be most effective in immunotherapy, include:

Arg-Asp-Lys-Asp-Val-Tyr-Arg

Lys-Asp-Lys-Asp-Val-Tyr-Lys

Lys-Glu-Lys-Asp-Val-Tyr-Lys

Arg-Glu-Arg-Asp-Val-Tyr-Arg

Lys-Glu-Leu-Tyr-Arg-Lys-Glu

Lys-Glu-Leu-Glu-Lys-Lys-Glu

Arg-Glu-Leu-Glu-Arg-Arg-Glu

Lys-Asp-Val-Asp-Lys-Lys-Asp

Lys-Asp-Leu-Glu-Lys-Lys-Glu

Tyr-Arg-Lys-Asp-Val-Tyr-Arg-Lys-Glu

Tyr-Arg-Lys-Glu-Leu-Glu-Lys-Lys-Glu

Glu-Lys-Lys-Glu-Leu-Tyr-Arg-Lys-Glu

Tyr-Arg-Lys-Glu-Leu-Tyr-Arg-Glu-Lys

Tyr-Arg-Lys-Asp-Val-Tyr-Arg-Lys-Asp-Val-Tyr-Arg

Glu-Lys-Lys-Glu-Leu-Tyr-Arg-Lys-Glu-Leu-Tyr-Arg

Glu-Arg-Lys-Glu-Leu-Tyr-Arg-Lys-Glu-Leu-Tyr-Arg

Examples of peptides synthesized in accordance with the present invention, which have been found to be

most effective in immunotherapy, include:

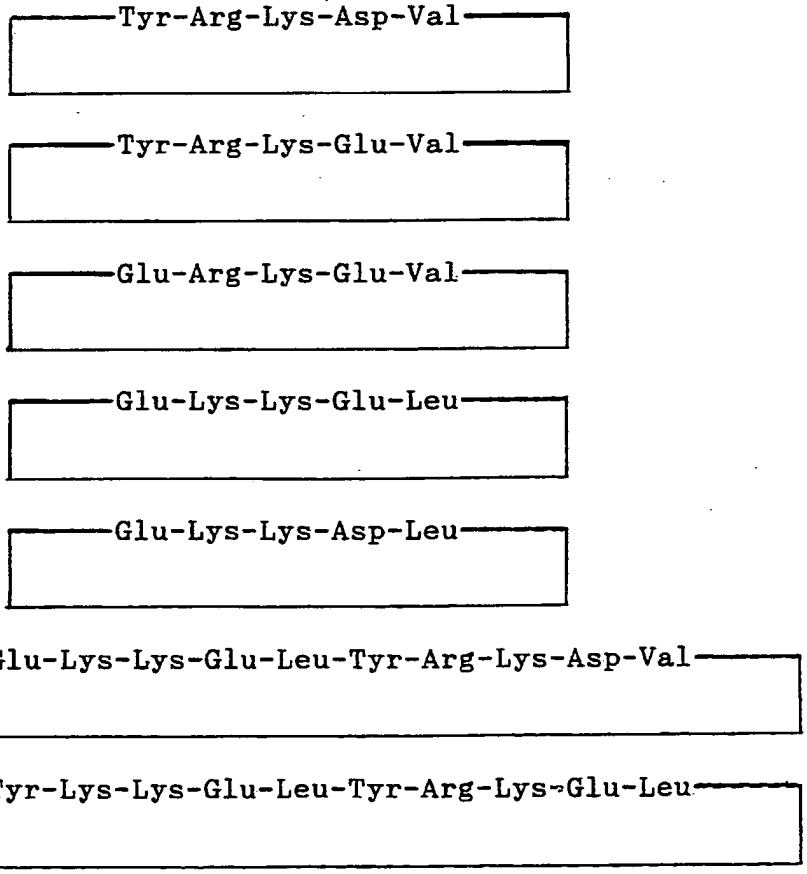
Glu-Arg-Lys-Glu-Leu-Tyr-Arg

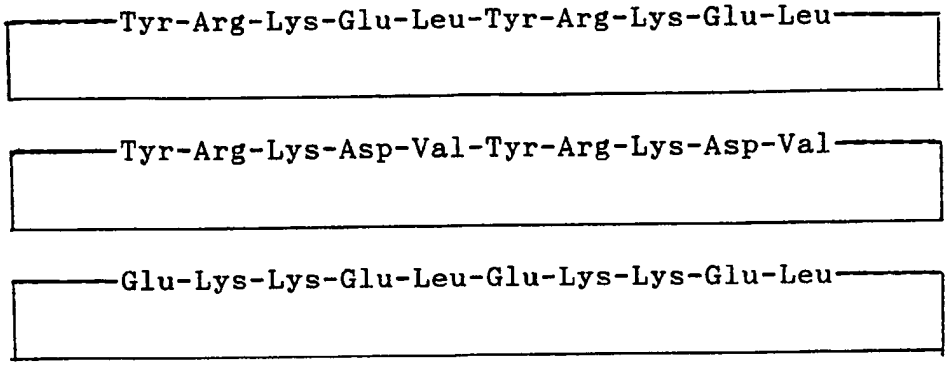
Tyr-Arg-Lys-Asp-Val-Tyr-Arg

Tyr-Arg-Lys-Glu-Leu-Tyr-Arg

Glu-Arg-Lys-Asp-Val-Tyr-Arg

Cyclized peptides synthesized in accordance with the present invention, and which are preferred for their effective in immunotherapy, include:





Other peptides encompassed within the scope of the present invention and which have effective levels of immunotherapeutic activity, though not as great as those peptides listed above, further include:

Arg-Lys-Asp-Val-Lys-Tyr;	Arg-Lys-Asp-Val-Arg-Tyr;
Arg-Lys-Asp-Val-Phe-Glu;	Arg-Glu-Glu-Val-Phe-Glu;
Arg-Lys-Asp-Val-Tyr-Arg;	Arg-Asn-Asp-Val-Tyr-Arg;
Arg-Lys-Glu-Leu-Tyr-Arg;	Arg-Gln-Glu-Leu-Tyr-Arg;
Arg-Lys-Asp-Val-Tyr-Lys;	Arg-Asn-Asp-Val-Tyr-Lys;
Arg-Lys-Glu-Leu-Tyr-Lys;	Arg-Gln-Glu-Leu-Tyr-Lys;
Arg-Lys-Asp-Val-Glu-Arg;	Arg-Lys-Glu-Leu-Glu-Arg;
Arg-Lys-Glu-Val-Glu-Arg;	Arg-Phe-Leu-Tyr-Arg-Asp;
Phe-Tyr-Arg-Leu-Arg-Tyr;	Glu-Tyr-Arg-Leu-Arg-Tyr;
Glu-Tyr-Arg-Leu-Tyr-Arg;	Lys-Glu-Leu-Gln-Glu-Glu;
Lys-Asp-Val-Arg-Tyr-Tyr;	Lys-Asp-Val-Lys-Phe-Glu;
Lys-Asp-Val-Arg-Phe-Glu;	Lys-Asp-Val-Arg-Tyr-Asp;

Arg-Tyr-Asn-Val-Tyr-Arg; Arg-Tyr-Gln-Leu-Tyr-Arg;

Arg-Tyr-Asn-Val-Tyr-Lys; Arg-Tyr-Gln-Leu-Tyr-Lys;

Arg-Glu-Lys-Leu-Tyr-Arg; Gln-Glu-Lys-Leu-Tyr-Arg;

Gln-Asp-Lys-Leu-Tyr-Arg; Asn-Asp-Lys-Leu-Tyr-Arg;

Lys-Tyr-Val-Tyr-Arg-Asp; Tyr-Arg-Asp-Val-Tyr-Arg;

Tyr-Lys-Asp-Val-Tyr-Lys; Tyr-Lys-Glu-Leu-Tyr-Arg;

Asp-Asn-Tyr-Val-Tyr-Arg; Asp-Asn-Tyr-Leu-Glu-Arg;

Asp-Asn-Tyr-Leu-Glu-Gln; Arg-Asn-Lys-Asp-Val-Tyr-Arg;

Arg-Gln-Arg-Glu-Leu-Tyr-Arg;

Arg-Asn-Arg-Tyr-Leu-Tyr-Arg;

Arg-Asp-Val-Tyr-Arg-Gln-Asn;

Lys-Asp-Val-Tyr-Arg-Gln-Asn;

Arg-Asp-Val-Tyr-Lys-Gln-Asn;

Asp-Glu-Lys-Glu-Leu-Tyr-Arg;

Glu-Glu-Lys-Asp-Val-Tyr-Arg;

Asp-Glu-Gln-Glu-Leu-Phe-Arg;

Gln-Asp-Val-Tyr-Arg-Glu-Asp;

Gln-Glu-Asn-Asp-Val-Glu-Gln;

Asn-Asp-Gln-Glu-Leu-Glu-Gln;

Asn-Asp-Lys-Asp-Val-Asp-Lys;

Gln-Glu-Asp-Lys-Leu-Tyr-Arg;

Arg-Glu-Asp-Lys-Leu-Tyr-Arg;

Arg-Glu-Asp-Arg-Leu-Tyr-Arg;

Arg-Asp-Glu-Arg-Leu-Tyr-Arg;

Arg-Glu-Tyr-Arg-Leu-Tyr-Arg;

Lys-Glu-Tyr-Arg-Leu-Tyr-Arg;

Lys-Asp-Val-Tyr-Arg-Arg-Tyr;

Lys-Asp-Val-Tyr-Arg-Lys-Tyr;

Lys-Asp-Val-Tyr-Arg-Lys-Asp;

Lys-Glu-Leu-Glu-Arg-Lys-Glu;

Arg-Asp-Val-Asp-Lys-Lys-Asp;

Lys-Asp-Leu-Glu-Lys-Lys-Asp;

Lys-Glu-Leu-Lys-Glu-Lys-Glu;

Lys-Asp-Leu-Lys-Glu-Lys-Asp;

Glu-Arg-Lys-Asp-Val-Tyr-Arg;

Glu-Lys-Lys-Asp-Val-Tyr-Arg;

Asp-Arg-Lys-Asp-Val-Tyr-Arg;

Lys-Glu-Leu-Lys-Glu-Tyr-Arg;

Lys-Asp-Val-Lys-Glu-Tyr-Lys;

Glu-Lys-Lys-Glu-Leu-Glu-Lys-Lys-Glu;

Glu-Lys-Lys-Glu-Leu-Tyr-Arg-Arg-Tyr;

Glu-Lys-Lys-Glu-Leu-Arg-Tyr-Tyr-Arg;

Tyr-Arg-Lys-Asp-Val-Lys-Tyr-Tyr-Arg;

Glu-Lys-Lys-Glu-Leu-Tyr-Arg-Tyr-Arg;

Glu-Lys-Lys-Glu-Leu-Tyr-Arg-Glu-Arg;

Glu-Lys-Lys-Glu-Leu-Tyr-Arg-Glu-Arg;

Glu-Lys-Lys-Glu-Leu-Glu-Gln-Tyr-Arg;

Glu-Lys-Lys-Glu-Leu-Glu-Gln-Asp-Asn;

Lys-Glu-Glu-Lys-Leu-Glu-Lys-Lys-Glu;

Lys-Asp-Asp-Lys-Leu-Tyr-Arg-Gln-Glu;

Gln-Glu-Asp-Lys-Leu-Tyr-Arg-Gln-Glu;

Gln-Glu-Asp-Lys-Leu-Tyr-Arg-Gln-Glu;

Arg-Tyr-Leu-Arg-Tyr-Leu-Tyr-Arg;

Arg-Tyr-Leu-Lys-Glu-Leu-Tyr-Arg;

Tyr-Arg-Gly-Lys-Glu-Leu-Tyr-Arg;

Arg-Tyr-Leu-Tyr-Arg-Lys-Asp-Val-Tyr-Arg; and,

Arg-Tyr-Leu-Glu-Lys-Lys-Glu-Leu-Tyr-Arg.

Linear peptides of the present invention may be synthesized via procedures generally known to those skilled in the art and which are of wide use at present. M. Bodanszky and A. Bodanszky, Peptide Chemistry: A Practical Textbook, Springer-Verlag, N.Y. (1988); M. Bodanszky and A. Bodanszky, The Practice of Peptide Synthesis, Springer-Verlag, N.Y. (1989). All linear peptides of the present invention, for example, may be synthesized by the solid phase method utilizing peptide synthesizers which are commercially available through, for example, Applied Biosystems, Inc., Foster City,

California, U.S.A., and t-Boc chemistry.

In practice, crude preparations of peptides were purified by low pressure column ion-exchange chromatography following preparative ion-exchange as reverse phase HPLC. Purity of the final products had been analyzed by analytical reverse phase HPLC using ODS-columns and structure was verified by amino acid analysis. Purity of peptides synthesized varied from 94.2% to 99.2%.

Cyclized peptides synthesized in accordance with the present invention may be synthesized in accordance with the following:

Dicarbonic amino acids were attached to a resin via estification of a side chain carboxyl group and deprotected. Aminoacid-O-nitrophenol esters and aminoacid-O-succinimide esters were coupled directly to the deprotected alpha-amino groups of the growth peptide in the desired sequence to produce C-terminal and N-terminal deprotected peptide-resin. These resin-bounded peptides were cyclized using dicyclohexyl carbodiimide, cleaved and side chain deprotected following purification. Aminoacidic

analysis or NMR spectra may be utilized to confirm that the desired cyclized peptide has been produced.

Other objects and features of the present invention will be described in connection with the accompanying drawing figures and tables, which further illustrate the invention. It should, however, be recognized that the accompanying figures and tables are intended solely to illustrate the invention and are not intended as a means for defining the scope of the invention.

Brief Description of the Drawing Figures/Tables

Figures 1 - 5 (i.e., Tables 1 - 5) present experimental data illustrating the use and effectiveness of the peptides of the present invention.

Detailed Description of the Preferred Embodiments

Turning now, in detail, to an analysis of the preferred embodiments and experimental data, conducted by the inventor, which illustrates the immunotherapeutic activity of the peptide compounds of the present invention.

Initially, a suboptimal amount of phyto-

hemagglutinin from Phaseolus vulgaris was utilized in experimentation. Phytohemagglutinin is a non-specific stimulator of T-lymphocyte activation, which does not induce ^{3}H-thymidine uptake by peripheral blood lymphocytes in sub-optimal amounts. Peptides of the present invention were added in a wide range of concentrations to lymphocyte cultures and caused a dramatic increase of ^{3}H-thymidine incorporation into cell DNA.

As shown in Table 1, the peptides of the invention are over 100 to 1000 times more active than other known immunologically-active sequences. The peptides tested in Table 1 were as follows:

Peptides	Amino Acid Sequence
Thymopoietin NN32-36	Arg-Lys-Asp-Val-Tyr
Thymosin alpha-1 N23-27	Val-Glu-Glu-Ala-Glu
Peptide (a)	Arg-Lys-Asp-Val-Tyr-Arg
Peptide (b)	Arg-Lys-Asp-Val-Tyr-Lys
Peptide (c)	Glu-Arg-Lys-Asp-Val-Tyr-Arg
Peptide (d)	Tyr-Arg-Lys-Asp-Val-Tyr-Arg

Peripheral blood lymphocytes (PBL) were purified by isopicnic centrifugation, washed and diluted in

complete RPMI 1640 medium, supplemented with human AB sera. 10^5 cells were added into the wells of 96 well plates, which contain 100 µl of tested substances, diluted in RPMI 1640 culture medium as above, and a suboptimal amount of PHA. Cells were cultured for 4 days and ^{3}H-thymidine was added into each well for 24 hours. Cells were harvested and incorporation of ^{3}H-thymidine was measured by scintillation counting. All experiments were conducted in quadruplicate.

The addition of peptides to the peripheral blood lymphocytes of patients with depressed immunity, as reflected by decreased expression of T-cell subset markers and lymphocyte deviations, normalizes these characteristics. Positive results were achieved within one hour of incubation, while no normalization was seen after such time when any presently known sequence was added. By contrast, previously known sequences led to limited normalization, but only after 18 - 24 hours of incubation. The experimental data of such comparative testing is set forth in Table 2.

With respect to the data set forth in Table 2, the PBL of normal persons and patients were treated

by the peptide Glu-Lys-Lys-Asp-Val-Tyr-Arg or Thymosin alpha-1 fragment N23-27 in culture medium for either 1 hour or 24 hours. Expression of CD4, CD8 and NK markers was detected by immunofluorescene as being the number of positive cells in cultures. All experiments were conducted in triplicate. P was calculated by using the *t* distribution of Student.

As measured by ^{3}H-thymidine uptake, the peptides of the invention were able to restore normal response to phytohemagglutinin, while the amino acid sequences of peptides known to the prior art failed to do so. The experimental results are presented in Table 3. (Such comparative testing was performed in a manner similar to that described above for the test results set forth in Table 1. PBL of patients with signs of immunodeficiency were tested for PHA-induced ^{3}H-thymidine uptake in the presence of tested substances in culture medium.)

It is known that <u>vaccinia</u> virus partially protects the mouse from infection with <u>Ectromelia</u> virus. The latter infection is lethal in most cases in inbred mice. Mice BALB/c received peptides of the present invention in doses of from 0.1 mcg to 1 mcg

per kg of body weight, one day prior to exposure to vaccinia virus. A month later, they were injected with Ectromelia virus in an extract from the spleen and liver of spontaneously infected animals. The clinical signs of infection on record were hepatosplenomegaly, mucosal inflammation and necrosis of extremities and tail. The potentiating effect of peptides following vaccination with viccinia virus was evident. The test results are presented in Table 4 for the peptide Tyr-Arg-Lys-Glu-Leu-Tyr-Arg versus a placebo. (In Table 4, P was calculated using the "F" distribution of Fischer, also known as Fischer's exact test.)

A single injection of a peptide one day prior to intranasal application in mice C57BL/6 with Influenza A virus strain WSN saved a significant number of animals. (See, Table 5.) The peptide had the amino acid sequence of Tyr-Arg-Lys-Glu-Leu-Glu-Lys-Lys-Glu. Mortality was recorded during a two-week period. P was calculated using the "F" distribution of Fischer.

Peptides of the present invention, injected in doses of from 0.1 mcg to 1 mcg per kg of mouse body weight, normalized the depleted T-cell immunity of

thymectomized animals.

In toxicity studies, no toxicity was observed for the peptides of the invention in doses of up to 1 g per kg of mouse body weight; 20 subcutaneous or intraperitoneal injections every other day of the same dose also failed to produce any signs of toxicity. There were no significant changes in arterial blood pressure, heart rate, respiration or body temperature between the placebo group and animals injected with peptides during acute or chronic toxicity studies. The peptides of the invention did not cause any morphological changes in the brain, heart, lung, kidney and liver tissues. Complete blood counts on the experimental animals revealed an elevation of white blood cells to high normal levels. Hyperplasia of the thymus and thymus-dependent zones of lymph nodes was recorded.

The medical formulations of the present invention preferably include an effective amount of one or more peptides and a solubilizer, with the possible inclusion of additional carriers and preservatives, as determined by the specifics of the different product formulations so desired by the

skilled artisan.

Solubilizers useful in combination with the peptides of the present invention include any solubilizer which is compatible with bodily fluids. Examples of solubilizers are water, solvents such as dimethylsulfoxide, propylene glycol, dimethylformamide and mixtures thereof, and surface active agents, such as non-ionic alkylene oxide block copolymers.

When the solubilizer used is water, additional additives can be used to achieve physiological concentrations of inorganic salts, normal osmotic pressure and effective lyophilization. Such additives can be selected from, for example, sodium chloride and potassium chloride, sodium and potassium phosphate salts, sucrose, glucose, protein hydrolysate, dextran, polyvinylpyrrolidone and polyethylene glycol, among others.

In accordance with a preferred embodiment, peptides are included in medical treatment composition of the present invention in order to provide doses ranging from 0.0001 mg to about 5 mg

per kg of body weight for parenteral use, as well as concentrations ranging from about 0.0001% to about 5% for topical use.

A preferred composition of the present invention for parenteral usage is a 0.01% solution of peptides in 1 ml of 0.85 wt-% sodium chloride containing 0.1 wt-% of ascorbic acid; it can be lyophilized in the presence of 100 mg glucose.

A preferred composition of the present invention for eye and ear drops, for example, is a composition comprising a 0.0001 wt-% solution of peptides in a 0.85% sodium chloride solution.

The medicinal compositions utilizing the peptides of the present invention may be used for immunomodulation of various immunodeficiencies and immunosuppressed conditions, T-cell subset and lymphocyte deviations, enhancement of a vaccine's efficacy, as well as for immunotherapy, including infections, local or systemic complications of non-infectious diseases, postoperatives inflammations, wounds and burns.

While only several embodiments of the present invention have been described, it will be obvious to those of ordinary skill in the art that many modifications may be made to the present invention without departing from the spirit and scope thereof.

Claims

1. A peptide compound, or an acid or base salt thereof, constructed by combination and/or overlapping of the amino acid sequences:

A_1B_1 X B_2A_2, A_3B_3 X A_4B_4, B_5A_5 X A_6B_6,

B_7A_7 X B_8A_8, A_9B_9, $A_{10}A_{11}$,

$B_{10}A_{12}$ and/or $B_{11}B_{12}$,

wherein,

X is a hydrophobic or neutral amino acid independently selected from the group consisting of Ala, *D*-Ala, Gly, *D*-Gly, Ile, *D*-Ile, Leu, *D*-Leu, Met, *D*-Met, Phe, *D*-Phe, Pro, *D*-Pro, Sar, *D*-Sar, Ser, *D*-Ser, Tre, *D*-Tre, Trp, *D*-Trp, Val and *D*-Val;

A_1 through A_{12} are, independently, neutral or positively-side-chain-charged amino acids independently selected from the group consisting of Ala, *D*-Ala, Arg, *D*-Arg, Asp, *D*-Asp, Glu, *D*-Glu, Gly, *D*-Gly, His, *D*-His, Ile, *D*-Ile, Leu, *D*-Leu, Met, *D*-Met, Phe, *D*-Phe, Pro, *D*-Pro, dehydro Pro, Sar, *D*-Sar, Ser, *D*-Ser, Tre, *D*-Tre,

Trp, *D*-Trp, Val and *D*-Val; and,

B_1 through B_{12} are, independently, neutral or negatively-side-chain-charged amino acids independently selected from the group consisting of Ala, *D*-Ala, Asp, *D*-Asp, Glu, *D*-Glu, Gly, *D*-Gly, Ile, *D*-Ile, Leu, *D*-Leu, Met, *D*-Met. Phe, *D*-Phe, Pro, *D*-Pro, dehydro Pro, Sar, *D*-Sar, Tre, *D*-Tre, Tyr, *D*-Tyr, Val, *D*-Val, *L*-2- amino-glutaryl, *D*-2-aminoglutaryl, *L*-2-aminoadiphyl, *D*-2-aminoadiphyl, *L*-2-aminopimelyl and *D*-2-aminopimelyl.

2. The peptide compound according to Claim 1, wherein,

X is an amino acid selected from the group consisting of Ala, Gly, Ile, Leu, Phe and Val;

A_1 through A_n are amino acids independently selected from the group consisting of Arg, Asn, Gln, Lys, Phe and Val; and,

B_1 through B_n are amino acids independently selected from the group consisting of Asp, Glu, Tyr, Phe and Val.

3. The peptide compound according to Claim 1, wherein said peptide compound has balanced side chain charges of amino acid sequences which are symmetrical to X.

4. The peptide compound according to Claim 1, wherein said peptide compound has uncompensated opposite side chain charges of amino acid sequences relative to X.

5. The peptide compound according to Claim 1, wherein said peptide compound is Glu-Arg-Lys-Glu-Leu-Tyr-Arg.

6. The peptide compound according to Claim 1, wherein said peptide compound is Tyr-Arg-Lys-Asp-Val-Tyr-Arg.

7. The peptide compound according to Claim 1, wherein said peptide compound is Tyr-Arg-Lys-Glu-Leu-Tyr-Arg.

8. The peptide compound according to Claim 1, wherein said peptide compound is Glu-Arg-Lys-Asp-Val-Tyr-Arg.

9. The peptide compound according to Claim 1, wherein said peptide compound is a member selected from the group consisting of:

Arg-Asp-Lys-Asp-Val-Tyr-Arg;

Lys-Asp-Lys-Asp-Val-Tyr-Lys;

Lys-Glu-Lys-Asp-Val-Tyr-Lys;

Arg-Glu-Arg-Asp-Val-Tyr-Arg;

Lys-Glu-Leu-Tyr-Arg-Lys-Glu;

Lys-Glu-Leu-Glu-Lys-Lys-Glu;

Arg-Glu-Leu-Glu-Arg-Arg-Glu;

Lys-Asp-Val-Asp-Lys-Lys-Asp;

Lys-Asp-Leu-Glu-Lys-Lys-Glu;

Tyr-Arg-Lys-Asp-Val-Tyr-Arg-Lys-Glu;

Tyr-Arg-Lys-Glu-Leu-Glu-Lys-Lys-Glu;

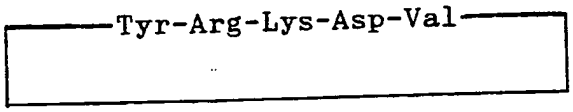
Glu-Lys-Lys-Glu-Leu-Tyr-Arg-Lys-Glu;

Tyr-Arg-Lys-Glu-Leu-Tyr-Arg-Glu-Lys;

Tyr-Arg-Lys-Asp-Val-Tyr-Arg-Lys-Asp-Val-Tyr-Arg;

Glu-Lys-Lys-Glu-Leu-Tyr-Arg-Lys-Glu-Leu-Tyr-Arg;

Glu-Arg-Lys-Glu-Leu-Tyr-Arg-Lys-Glu-Leu-Tyr-Arg;



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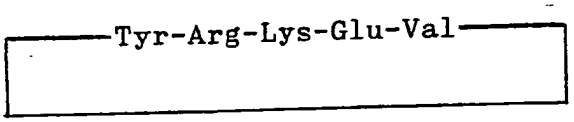

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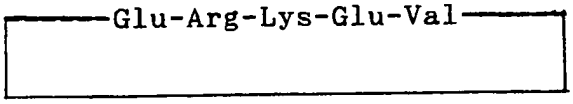
22 

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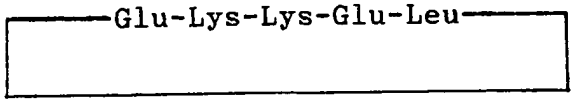
23 

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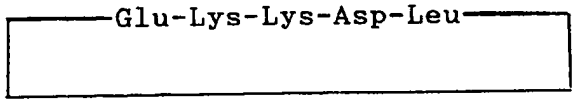
24 

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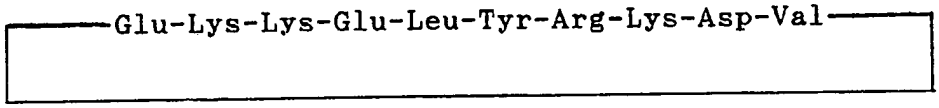
25 

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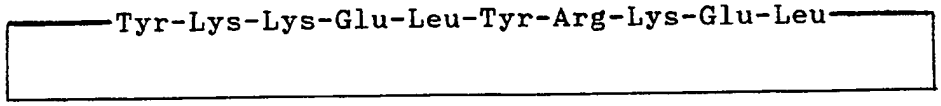
26 

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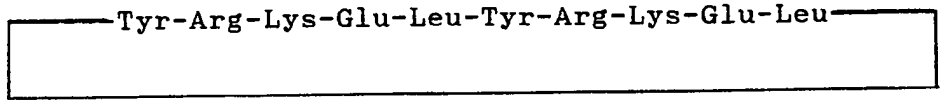
27 

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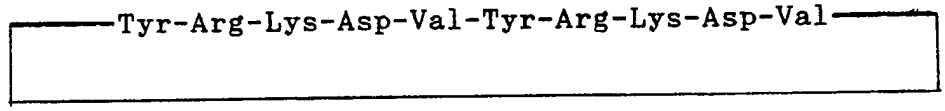
28 

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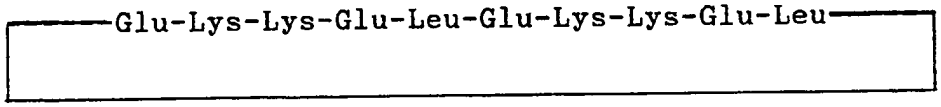
30 

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10. The peptide compound according to Claim 1, wherein said peptide compound is a member selected from the group consisting of:

Arg-Lys-Asp-Val-Lys-Tyr; Arg-Lys-Asp-Val-Arg-Tyr;

5 Arg-Lys-Asp-Val-Phe-Glu; Arg-Glu-Glu-Val-Phe-Glu;

Arg-Lys-Asp-Val-Tyr-Arg; Arg-Asn-Asp-Val-Tyr-Arg;

Arg-Lys-Glu-Leu-Tyr-Arg; Arg-Gln-Glu-Leu-Tyr-Arg;

Arg-Lys-Asp-Val-Tyr-Lys; Arg-Asn-Asp-Val-Tyr-Lys;

Arg-Lys-Glu-Leu-Tyr-Lys; Arg-Gln-Glu-Leu-Tyr-Lys;

Arg-Lys-Asp-Val-Glu-Arg; Arg-Lys-Glu-Leu-Glu-Arg;

Arg-Lys-Glu-Val-Glu-Arg; Arg-Phe-Leu-Tyr-Arg-Asp;

Phe-Tyr-Arg-Leu-Arg-Tyr; Glu-Tyr-Arg-Leu-Arg-Tyr;

Glu-Tyr-Arg-Leu-Tyr-Arg; Lys-Glu-Leu-Gln-Glu-Glu;

Lys-Asp-Val-Arg-Tyr-Tyr; Lys-Asp-Val-Lys-Phe-Glu;

Lys-Asp-Val-Arg-Phe-Glu; Lys-Asp-Val-Arg-Tyr-Asp;

Arg-Tyr-Asn-Val-Tyr-Arg; Arg-Tyr-Gln-Leu-Tyr-Arg;

Arg-Tyr-Asn-Val-Tyr-Lys; Arg-Tyr-Gln-Leu-Tyr-Lys;

Arg-Glu-Lys-Leu-Tyr-Arg; Gln-Glu-Lys-Leu-Tyr-Arg;

Gln-Asp-Lys-Leu-Tyr-Arg; Asn-Asp-Lys-Leu-Tyr-Arg;

Lys-Tyr-Val-Tyr-Arg-Asp; Tyr-Arg-Asp-Val-Tyr-Arg;

Tyr-Lys-Asp-Val-Tyr-Lys; Tyr-Lys-Glu-Leu-Tyr-Arg;

Asp-Asn-Tyr-Val-Tyr-Arg; Asp-Asn-Tyr-Leu-Glu-Arg;

Asp-Asn-Tyr-Leu-Glu-Gln; Arg-Asn-Lys-Asp-Val-Tyr-Arg;

Arg-Gln-Arg-Glu-Leu-Tyr-Arg;

Arg-Asn-Arg-Tyr-Leu-Tyr-Arg;

Arg-Asp-Val-Tyr-Arg-Gln-Asn;

Lys-Asp-Val-Tyr-Arg-Gln-Asn;

Arg-Asp-Val-Tyr-Lys-Gln-Asn;

Asp-Glu-Lys-Glu-Leu-Tyr-Arg;

Glu-Glu-Lys-Asp-Val-Tyr-Arg;

Asp-Glu-Gln-Glu-Leu-Phe-Arg;

Gln-Asp-Val-Tyr-Arg-Glu-Asp;

Gln-Glu-Asn-Asp-Val-Glu-Gln;

Asn-Asp-Gln-Glu-Leu-Glu-Gln;

Asn-Asp-Lys-Asp-Val-Asp-Lys;

Gln-Glu-Asp-Lys-Leu-Tyr-Arg;

Arg-Glu-Asp-Lys-Leu-Tyr-Arg;

Arg-Glu-Asp-Arg-Leu-Tyr-Arg;

Arg-Asp-Glu-Arg-Leu-Tyr-Arg;

Arg-Glu-Tyr-Arg-Leu-Tyr-Arg;

Lys-Glu-Tyr-Arg-Leu-Tyr-Arg;

Lys-Asp-Val-Tyr-Arg-Arg-Tyr;

Lys-Asp-Val-Tyr-Arg-Lys-Tyr;

Lys-Asp-Val-Tyr-Arg-Lys-Asp;

Lys-Glu-Leu-Glu-Arg-Lys-Glu;

Arg-Asp-Val-Asp-Lys-Lys-Asp;

Lys-Asp-Leu-Glu-Lys-Lys-Asp;

Lys-Glu-Leu-Lys-Glu-Lys-Glu;

Lys-Asp-Leu-Lys-Glu-Lys-Asp;

Glu-Arg-Lys-Asp-Val-Tyr-Arg;

Glu-Lys-Lys-Asp-Val-Tyr-Arg;

Asp-Arg-Lys-Asp-Val-Tyr-Arg;

Lys-Glu-Leu-Lys-Glu-Tyr-Arg;

Lys-Asp-Val-Lys-Glu-Tyr-Lys;

Glu-Lys-Lys-Glu-Leu-Glu-Lys-Lys-Glu;

Glu-Lys-Lys-Glu-Leu-Tyr-Arg-Arg-Tyr;

Glu-Lys-Lys-Glu-Leu-Arg-Tyr-Tyr-Arg;

Tyr-Arg-Lys-Asp-Val-Lys-Tyr-Tyr-Arg;

Glu-Lys-Lys-Glu-Leu-Tyr-Arg-Tyr-Arg;

Glu-Lys-Lys-Glu-Leu-Tyr-Arg-Glu-Arg;

Glu-Lys-Lys-Glu-Leu-Tyr-Arg-Glu-Arg;

Glu-Lys-Lys-Glu-Leu-Glu-Gln-Tyr-Arg;

Glu-Lys-Lys-Glu-Leu-Glu-Gln-Asp-Asn;

Lys-Glu-Glu-Lys-Leu-Glu-Lys-Lys-Glu;

Lys-Asp-Asp-Lys-Leu-Tyr-Arg-Gln-Glu;

Gln-Glu-Asp-Lys-Leu-Tyr-Arg-Gln-Glu;

Gln-Glu-Asp-Lys-Leu-Tyr-Arg-Gln-Glu;

Arg-Tyr-Leu-Arg-Tyr-Leu-Tyr-Arg;

Arg-Tyr-Leu-Lys-Glu-Leu-Tyr-Arg;

Tyr-Arg-Gly-Lys-Glu-Leu-Tyr-Arg;

Arg-Tyr-Leu-Tyr-Arg-Lys-Asp-Val-Tyr-Arg; and,

Arg-Tyr-Leu-Glu-Lys-Lys-Glu-Leu-Tyr-Arg.

11. A composition having immunotheapeutic activity, comprising:

a peptide compound, or an acid or base salt thereof, constructed by combination and/or overlap-

ping of the amino acid sequences:

A_1B_1 X B_2A_2, A_3B_3 X A_4B_4, B_5A_5 X A_6B_6,

B_7A_7 X B_8A_8, A_9B_9, $A_{10}A_{11}$,

$B_{10}A_{12}$ and/or $B_{11}B_{12}$,

wherein,

X is a hydrophobic or neutral amino acid independently selected from the group consisting of Ala, *D*-Ala, Gly, *D*-Gly, Ile, *D*-Ile, Leu, *D*-Leu, Met, *D*-Met, Phe, *D*-Phe, Pro, *D*-Pro, Sar, *D*-Sar, Ser, *D*-Ser, Tre, *D*-Tre, Trp, *D*-Trp, Val and *D*-Val;

A_1 through A_{12} are, independently, neutral or positively-side-chain-charged amino acids independently selected from the group consisting of Ala, *D*-Ala, Arg, *D*-Arg, Asp, *D*-Asp, Glu, *D*-Glu, Gly, *D*-Gly, His, *D*-His, Ile, *D*-Ile, Leu, *D*-Leu, Met, *D*-Met, Phe, *D*-Phe, Pro, *D*-Pro, dehydro Pro, Sar, *D*-Sar, Ser, *D*-Ser, Tre, *D*-Tre, Trp, *D*-Trp, Val and *D*-Val; and,

B_1 through B_{12} are, independently, neutral or negatively-side-chain-charged amino acids

independently selected from the group consisting of Ala, *D*-Ala, Asp, *D*-Asp, Glu, *D*-Glu, Gly, *D*-Gly, Ile, *D*-Ile, Leu, *D*-Leu, Met, *D*-Met. Phe, *D*-Phe, Pro, *D*-Pro, dehydro Pro, Sar, *D*-Sar, Tre, *D*-Tre, Tyr, *D*-Tyr, Val, *D*-Val, *L*-2- amino-glutaryl, *D*-2-aminoglutaryl, *L*-2-aminoadiphyl, *D*-2-aminoadiphyl, *L*-2-aminopimelyl and *D*-2-aminopimelyl;

and,

a solubilizer for said peptide compound.

12. The composition having immunotherapeutic activity according to Claim 11, wherein said peptide compound is Glu-Arg-Lys-Glu-Leu-Tyr-Arg.

13. The composition having immunotherapeutic activity according to Claim 11, wherein said peptide compound is Tyr-Arg-Lys-Asp-Val-Tyr-Arg.

14. The composition having immunotherapeutic activity according to Claim 11, wherein said peptide compound is Tyr-Arg-Lys-Glu-Leu-Tyr-Arg.

15. The composition having immunotherapeutic activity according to Claim 11, wherein said peptide compound is Glu-Arg-Lys-Asp-Val-Tyr-Arg.

16. The composition having immunotherapeutic activity according to Claim 11, wherein said peptide compound is a member selected from the group consisting of:

Arg-Asp-Lys-Asp-Val-Tyr-Arg;

Lys-Asp-Lys-Asp-Val-Tyr-Lys;

Lys-Glu-Lys-Asp-Val-Tyr-Lys;

Arg-Glu-Arg-Asp-Val-Tyr-Arg;

Lys-Glu-Leu-Tyr-Arg-Lys-Glu;

Lys-Glu-Leu-Glu-Lys-Lys-Glu;

Arg-Glu-Leu-Glu-Arg-Arg-Glu;

Lys-Asp-Val-Asp-Lys-Lys-Asp;

Lys-Asp-Leu-Glu-Lys-Lys-Glu;

Tyr-Arg-Lys-Asp-Val-Tyr-Arg-Lys-Glu;

Tyr-Arg-Lys-Glu-Leu-Glu-Lys-Lys-Glu;

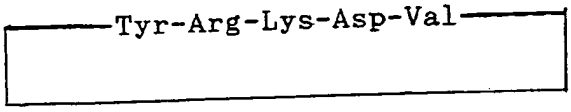
Glu-Lys-Lys-Glu-Leu-Tyr-Arg-Lys-Glu;

Tyr-Arg-Lys-Glu-Leu-Tyr-Arg-Glu-Lys;

Tyr-Arg-Lys-Asp-Val-Tyr-Arg-Lys-Asp-Val-Tyr-Arg;

Glu-Lys-Lys-Glu-Leu-Tyr-Arg-Lys-Glu-Leu-Tyr-Arg;

20 Glu-Arg-Lys-Glu-Leu-Tyr-Arg-Lys-Glu-Leu-Tyr-Arg;

21 

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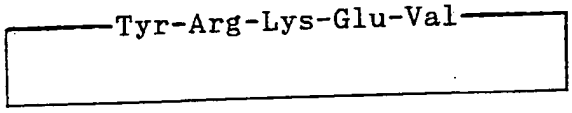
22 

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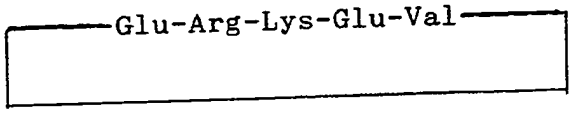
23 

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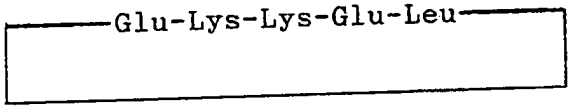
24 

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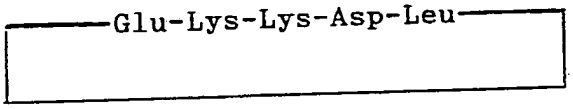
25 

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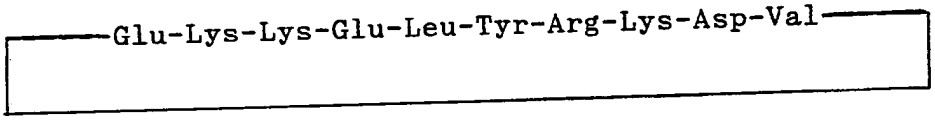
26 

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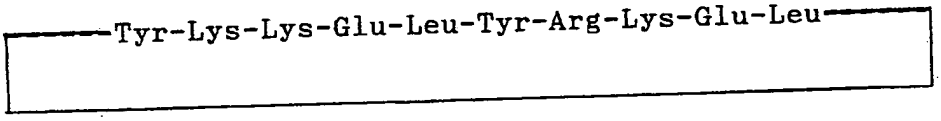
27 

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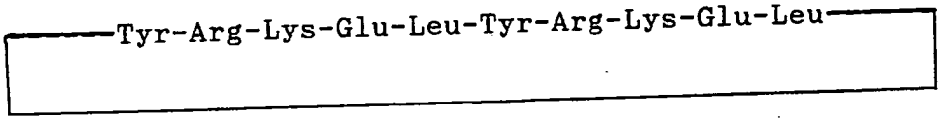
28 

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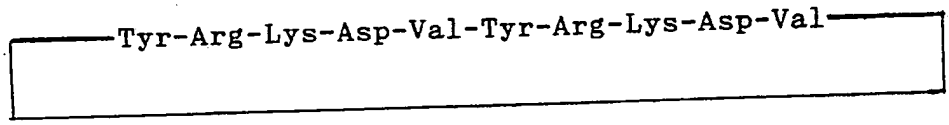
29 

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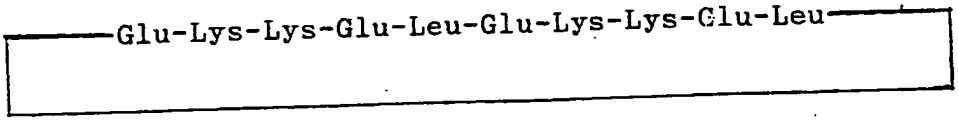
30 and,

31 

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17. The composition having immunotherapeutic activity according to Claim 11, wherein said peptide compound is a member selected from the group consisting of:

Arg-Lys-Asp-Val-Lys-Tyr; Arg-Lys-Asp-Val-Arg-Tyr;
Arg-Lys-Asp-Val-Phe-Glu; Arg-Glu-Glu-Val-Phe-Glu;
Arg-Lys-Asp-Val-Tyr-Arg; Arg-Asn-Asp-Val-Tyr-Arg;
Arg-Lys-Glu-Leu-Tyr-Arg; Arg-Gln-Glu-Leu-Tyr-Arg;
Arg-Lys-Asp-Val-Tyr-Lys; Arg-Asn-Asp-Val-Tyr-Lys;
Arg-Lys-Glu-Leu-Tyr-Lys; Arg-Gln-Glu-Leu-Tyr-Lys;
Arg-Lys-Asp-Val-Glu-Arg; Arg-Lys-Glu-Leu-Glu-Arg;
Arg-Lys-Glu-Val-Glu-Arg; Arg-Phe-Leu-Tyr-Arg-Asp;
Phe-Tyr-Arg-Leu-Arg-Tyr; Glu-Tyr-Arg-Leu-Arg-Tyr;
Glu-Tyr-Arg-Leu-Tyr-Arg; Lys-Glu-Leu-Gln-Glu-Glu;
Lys-Asp-Val-Arg-Tyr-Tyr; Lys-Asp-Val-Lys-Phe-Glu;
Lys-Asp-Val-Arg-Phe-Glu; Lys-Asp-Val-Arg-Tyr-Asp;
Arg-Tyr-Asn-Val-Tyr-Arg; Arg-Tyr-Gln-Leu-Tyr-Arg;
Arg-Tyr-Asn-Val-Tyr-Lys; Arg-Tyr-Gln-Leu-Tyr-Lys;
Arg-Glu-Lys-Leu-Tyr-Arg; Gln-Glu-Lys-Leu-Tyr-Arg;
Gln-Asp-Lys-Leu-Tyr-Arg; Asn-Asp-Lys-Leu-Tyr-Arg;
Lys-Tyr-Val-Tyr-Arg-Asp; Tyr-Arg-Asp-Val-Tyr-Arg;
Tyr-Lys-Asp-Val-Tyr-Lys; Tyr-Lys-Glu-Leu-Tyr-Arg;
Asp-Asn-Tyr-Val-Tyr-Arg; Asp-Asn-Tyr-Leu-Glu-Arg;
Asp-Asn-Tyr-Leu-Glu-Gln; Arg-Asn-Lys-Asp-Val-Tyr-Arg;
Arg-Gln-Arg-Glu-Leu-Tyr-Arg;

Arg-Asn-Arg-Tyr-Leu-Tyr-Arg;

Arg-Asp-Val-Tyr-Arg-Gln-Asn;

Lys-Asp-Val-Tyr-Arg-Gln-Asn;

Arg-Asp-Val-Tyr-Lys-Gln-Asn;

Asp-Glu-Lys-Glu-Leu-Tyr-Arg;

Glu-Glu-Lys-Asp-Val-Tyr-Arg;

Asp-Glu-Gln-Glu-Leu-Phe-Arg;

Gln-Asp-Val-Tyr-Arg-Glu-Asp;

Gln-Glu-Asn-Asp-Val-Glu-Gln;

Asn-Asp-Gln-Glu-Leu-Glu-Gln;

Asn-Asp-Lys-Asp-Val-Asp-Lys;

Gln-Glu-Asp-Lys-Leu-Tyr-Arg;

Arg-Glu-Asp-Lys-Leu-Tyr-Arg;

Arg-Glu-Asp-Arg-Leu-Tyr-Arg;

Arg-Asp-Glu-Arg-Leu-Tyr-Arg;

Arg-Glu-Tyr-Arg-Leu-Tyr-Arg;

Lys-Glu-Tyr-Arg-Leu-Tyr-Arg;

Lys-Asp-Val-Tyr-Arg-Arg-Tyr;

Lys-Asp-Val-Tyr-Arg-Lys-Tyr;

Lys-Asp-Val-Tyr-Arg-Lys-Asp;

Lys-Glu-Leu-Glu-Arg-Lys-Glu;

Arg-Asp-Val-Asp-Lys-Lys-Asp;

Lys-Asp-Leu-Glu-Lys-Lys-Asp;

Lys-Glu-Leu-Lys-Glu-Lys-Glu;

Lys-Asp-Leu-Lys-Glu-Lys-Asp;

Glu-Arg-Lys-Asp-Val-Tyr-Arg;

Glu-Lys-Lys-Asp-Val-Tyr-Arg;

Asp-Arg-Lys-Asp-Val-Tyr-Arg;

Lys-Glu-Leu-Lys-Glu-Tyr-Arg;

Lys-Asp-Val-Lys-Glu-Tyr-Lys;

Glu-Lys-Lys-Glu-Leu-Glu-Lys-Lys-Glu;

Glu-Lys-Lys-Glu-Leu-Tyr-Arg-Arg-Tyr;

Glu-Lys-Lys-Glu-Leu-Arg-Tyr-Tyr-Arg;

Tyr-Arg-Lys-Asp-Val-Lys-Tyr-Tyr-Arg;

Glu-Lys-Lys-Glu-Leu-Tyr-Arg-Tyr-Arg;

Glu-Lys-Lys-Glu-Leu-Tyr-Arg-Glu-Arg;

Glu-Lys-Lys-Glu-Leu-Tyr-Arg-Glu-Arg;

Glu-Lys-Lys-Glu-Leu-Glu-Gln-Tyr-Arg;

Glu-Lys-Lys-Glu-Leu-Glu-Gln-Asp-Asn;

Lys-Glu-Glu-Lys-Leu-Glu-Lys-Lys-Glu;

Lys-Asp-Asp-Lys-Leu-Tyr-Arg-Gln-Glu;

Gln-Glu-Asp-Lys-Leu-Tyr-Arg-Gln-Glu;

Gln-Glu-Asp-Lys-Leu-Tyr-Arg-Gln-Glu;

Arg-Tyr-Leu-Arg-Tyr-Leu-Tyr-Arg;

Arg-Tyr-Leu-Lys-Glu-Leu-Tyr-Arg;

Tyr-Arg-Gly-Lys-Glu-Leu-Tyr-Arg;

Arg-Tyr-Leu-Tyr-Arg-Lys-Asp-Val-Tyr-Arg; and,

Arg-Tyr-Leu-Glu-Lys-Lys-Glu-Leu-Tyr-Arg.

18. The composition having immunotherapeutic activity according to Claim 11, wherein said peptide compound is incorporated into a polymeric matrix selected from the group consisting of a polyanhydride copolymer, ethylen-vinyl acetate copolymer, lactic acid-glycolic acid copolymer, polyhydroxymethyl-metacrilate, polyvinyl alcohol and a combination thereof.

19. The composition having immunotherapeutic activity according to Claim 11, wherein said solubilizer is a member selected from the group consisting of water, dimethylsulfoxide, propylene glycol, dimethylformamide and a combination thereof.

20. The composition having immunotherapeutic activity according to Claim 11, wherein said solubilizer is a non-ionic alkylene oxide block copolymer.

21. The composition having immunotherapeutic activity according to Claim 11, further comprising an additive selected from the group consisting of a stabilizer, a viscosifier, a preservative and a combination thereof.

FIG.1 (TABLE 1)

3H-THYMIDINE UPTAKE BY DONOR'S LYMPHOCYTES IN (CPM $\bar{x}$ 10^3)
IN SUBOPTIMAL DOSES OF PHA

TESTED SUBSTANCES*

DOSE OF TESTED SUBSTANCE mcg/ml	THYMO-POIETIN NN 32-36	ALPHA 1 THYMOSIN N 23-27	PEPTIDE a	PEPTIDE b	PEPTIDE c	PEPTIDE d
0	0.6 ±0.2	0.5 ± 0.2	0.5 ± 0.2	0.4 ± 0.2	0.3 ± 0.1	0.6 ± 0.1
0.001	0.4 ±0.1	0.5 ± 0.2	1.8 ± 0.3	1.7 ± 0.2	2.1 ± 0.3	3.1 ± 0.3
0.01	0.5 ±0.1	0.6 ±0.1	2.5±0.3	3.6 ±0.3	4.2 ±0.3	4.0 ±0.2
0.1	0.5 ±0.2	0.4 ±0.1	3.2±0.3	3.2 ±0.3	4.0 ±0.3	4.3 ±0.3
1	0.8 ±0.3	0.6 ±0.2	3.6 ±0.4	3.4 ± 0.4	3.9 ±0.2	4.1 ± 0.3
10	0.9 ±0.2	0.9 ±0.2	3.4± 0.3	3.2 ±0.3	4.1 ± 0.2	4.1 ± 0.3
100	1.1 ±0.2	1.5 ± 0.3	3.0 ± 0.4	2.8 ±0.3	3.9 ± 0.3	4.2 ±0.3

* 3H THYMIDINE UPTAKE AT OPTIMAL CONCENTRATION OF PHA IS 4.1±0.3

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CORRECTION OF LYMPHOCYRE MARKER EXPRESSION IN VITRO

	MARKER		SHORT TERM CULTURE							
			PEPTIDE				THYMOSINE ALPHA-1 NN 23-27			
			1h		24h		1h		24h	
			% POSITIVE CELLS	P	%POSITIVE CELLS	P	%POSITIVE CELLS	P	%POSITIVE CELLS	P
DONOR	CD4	36.4 3±2	44.9 ± 2.2	<0.05	43.8 ±1.9	<0.05	37.4 ±2.4	70.5	38.1 ±1.9	> 0.2
	CD8	21.7 2±4	27.6 ± 1.7	<0.05	28.1 3.0	< 0.05	20.1 ±2.3	>0.2	23.2 2.7	> 0.2
	4|8 ratio	1.68	1.62	—	1.56	—	1.86	—	1.64	—
	NK	15.3 ± 2.1	25.1 ± 1.5	< 0.005	26.1±1.8	< 0.005	14.9 ±3.1	>0.2	20.4 ±2.0	< 0.05
ONCOLOGIC PATIENT	CD4	33.3 2±1	37.4 ± 1.4	< 0.005	38.9±2.1	< 0.005	34.1 ± 3.1	> 0.2	37.8 ±2.2	> 0.05
	CD8	29.5 1±4	19.7 ±2.3	< 0.005	20.1 ± 1.8	<0.005	29.9 ±2.5	> 0.2	26.1 ± 3.2	> 0.15
	4|8 ratio	1.13	1.89	—	1.91	—	1.14	—	1.45	—
	NK	14.1 ± 1.7	27.2 ± 3.1	<0.005	26.5 ± 2.3	< 0.005	14.4±2.9	> 0.2	24.4 ±1.8	<0.005
PATIENT WITH VIRAL INFECT-ION	CD4	26.8 2±2	33.8 ± 1.4	<0.01	35.2 ±2.3	< 0.02	26.3 ± 2.4	> 0.2	31.7 ± 2.8	> 0.05
	CD8	13.7 3±0	20.6 ± 1.7	0.05	21.9 ± 2.1	< 0.02	14.1 2.5	> 0.2	18.7 ± 2.4	> 0.05
	4|8 ratio	1.96	1.64	—	1.61	—	1.86	—	2.02	—
	NK	9.4 ± 1.4	25.7 ± 3.7	0.005	25.9 ±2.4	< 0.01	9.6 ± 2.1	70.2	21.3 ± 3.2	< 0.01

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FIG. 3 (TABLE)

IN VITRO CORRECTION OF ^{3}H-THYMIDINE UPTAKE BY PATIENTS' LYMPHOCYTES (CPM $\times 10^{3}$)

TESTED SUBSTANCE	DONORS		PATIENTS					
	L.K.	P.L.	M.A.	B.D.	K.E.	H.F.	O.D.	H.S.
CONTROL	1.34 ± 0.31	1.82 ± 0.19	0.78 ± 12	0.62 ± 0.21	0.80 ± 0.11	0.86 ± 0.10	0.52 ± 0.21	0.63 ± 0.2
THYMOPOIETIN NO. 32-36	1.51 ± 0.23	1.77 ± 0.16	0.89 ± 0.11	0.61 ± 0.12	1.23 ± 0.09	0.97 ± 0.12	0.91 ± 0.15	0.75 ± 0.16
ALPHA-L-THYMO-SINE NO.23 – 27	1.39 ± 0 21	1.73 ± 0.16	0.89 ± 0.13	0.71 ± 0.18	0.76 ± 0.11	0.94 ± 0.14	0.74 ± 0.14	0.97 ± 0.13
PEPTIDE	2.32 ± 0.24	2.12 ± 0.18	1.31 ± 0.14	2.09 ± 0.18	2.30 ± 0.19	1.75 ± 0.17	1.22 ± 0.13	1.42 ± 0.10

PHA WAS ADDED IN OPTIMAL CONCENTRATION, CPM WITHOUT PHA 0.21 ± 0.09.
ALL SUBSTANCES IN CONCENTRATIONS OF 1 mcg/ml
ALL EXPERIMENTS DONE IN TRIPLICETS
PEPTIDE HAS A SEQUENCE: TYR ARG LYS ASP VAL TYR ARG

FIG. 4 (TABLE 4)

ENHANCEMENT OF VACCINATION EFFECT AGAINST ECTROMELIA VIRUS

	SIGNS OF INFECTION	MORTALITY
PLACEBO	10/10	8/10
PEPTIDE	2/10	0/20
p	< 0.00036	< 0.00036

FIG. 5 (TABLE 5)

THE EFFECT OF PEPTIDES ON MOUSE MORTALITY INDUCED BY INFLUENZA A VIRUS

	INFLUENZA A VIRUS, LD_{100}			
	0	1	5	10
CONTROL	$^{0}/_{10}$	$^{9}/_{10}$	$^{10}/_{10}$	$^{10}/_{10}$
PEPTIDE	$^{0}/_{10}$	$^{0}/_{9}$	$^{2}/_{10}$	$^{6}/_{10}$
p	N.A.	< 0.0001	< 0.0001	< 0.043

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/08795

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): C07K 7/06,7/08,7/48; A61K 37/02
US CL : 514/15,16,17; 530/327,328,329

II. FIELDS SEARCHED

Minimum Documentation Searched[4]

Classification System	Classification Symbols
U.S.	514/15,16,17; 530/327,328,329

Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched[6]

APS, Biosis

III. DOCUMENTS CONSIDERED TO BE RELEVANT[14]

Category*	Citation of Document,[16] with indication, where appropriate, of the relevant passages[17]	Relevant to Claim No.[18]
A	US,A, 4,397,842 (Goldstein et al.) 09 August 1983, see entire document.	1-21
A	US, A, 4,910,296 (Birr et al) 20 March 1990, see entire document.	1-21
A	US, A, 4,002,740 (Goldstein et al) 11 January 1977, see entire document.	1-21
A	US, A, 4,659,694 (Horecker) 21 April 1987 see entire document.	1-21

* Special categories of cited documents:[16]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search[2]	Date of Mailing of this International Search Report[2]
24 FEBRUARY 1992	11 MAR 1992
International Searching Authority[1]	**Signature of Authorized Officer[20]**
ISA/US	S.G. Marshall

Form PCT/ISA/210 (second sheet)(May 1986) ß